Crystallization of proteins accompanied by formation of a cylindrical surface

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A possibility of the formation a self-contained cylindrical lattice under crystallization of proteins due to the anisotropic (trapezoidal) shape of their globules is discussed theoretically. The theory of cylindrical crystallization is considered. It is shown that in this case the list of the low-symmetry phases is completely determined by the symmetry of the cylindrical liquid, which is predetermined by the molecular shape. The phase symmetries and structures are found out. The phase diagrams are obtained. [S1063-651X(97)08208-1]

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In biological objects there is a set of examples of unusual structures arising under crystallization of certain proteins. Rather than crystallize into a bulk three-dimensional (3D) lattice, as usually occurs for all nonorganic and for most organic materials, these proteins form a self-contained cylindrical surface.

The most important example is microtubule formation under crystallization of the protein tubulin. This protein forms microtubules—long cylinders about 25 nm in outer diameter and 14–15 nm in inner diameter. They are constructed of tubulin dimers and have crystalline organization. Tubulin dimers align head to tail in 13 longitudinal parallel protofilaments, lining the wall of the microtubule. Each tubulin subunit is a polar dimer consisting of two slightly different tubulin α and β monomers, its polar character originating from calcium ions bounded within β monomers [1,2].

The proteins of the tobacco mosaic virus aggregate into a cylinder with an inner radius of about 200 nm and an outer one of about 800 nm and with a length of more than 30 000 nm with the RNA molecule lying along the helix. There is a region on the phase diagram in which the protein subunits are arranged in a helix [1].

Under crystallization of proteins of biological membranes formation of crystal patches is usually observed. Being more rigid, the protein crystal area is as a rule flattened with respect to the protein-free membrane region. However, in certain cases crystallization of proteins on initially spherical vesicles was followed by the transformation of vesicle shape into cylindrical shape, the vesicle surface being covered by a self-contained crystalline protein lattice [3].

In biological systems one can find already existing objects with cylindrically arranged proteins. In cylindrical giant cells of green water plants *Chara* and *Nitella* the actin filaments form a helical winding under the cell membrane, providing a helical stream of the cytoplasm [4].

The above examples stimulate interest in elucidating the reasons that proteins crystallize into a lattice, forming a closed cylindrical surface.

Crystallization of organic molecules and especially of proteins is often determined by gently sloping potentials, which enables us to apply in this case the theory of weak crystallization [5]. Crystallization should be considered weak if the transition from a liquid to a crystal is of the first order, but is rather close to the second order. In the case of 3D crystallization and 2D crystallization on a plane this imposes the rigid restriction that either it takes place in close vicinity to the isolated Curie point of the phase diagram or the cubic term is small for some reason [6]. It is shown in this paper that this limitation is absent under crystallization with the formation of the cylindrical surface.

Consider first a simple example of an aggregation of molecules whose shape in one of the three mutually perpendicular cross sections is trapezoidal and in the other two it is rectangular. Assume that under the aggregation the nearest molecules are oriented in the same way. These molecules form obviously an archlike surface with its radius fixed by the size of the trapezoid. On the one hand, in order to form a closed single-layer cylindrical surface the size of the molecules must be subject to certain geometrical conditions. However, if the molecules are elastic they can still form a cylindrical surface at any size of the trapezoid. On the other hand, formation of a multilayer cylindrical surface requires that some additional conditions on the subunits size be fulfilled, the elastic energy cost increasing with the number of layers under elastic adaptation. Hence the formation of a monolayer cylinder should be energetically favorable in some cases.

One can expect the globules of some proteins to have anisotropic shapes qualitatively corresponding to that discussed above [7]. The condition of the same orientation of proteins under aggregation can be realized for various reasons—because of either the orientational peculiarities of their interaction potentials or some geometric restrictions. The latter occurs, for example, under crystallization of proteins anchored onto one of the membrane sides [3].

When the anisotropy of protein globules is of the type discussed above one should expect a cylindrical surface to be formed under protein crystallization. The free energy describing this kind of crystallization involves the terms related to the protein conformational changes, elastic deformation and, in the case of membrane proteins, to the membrane shape change in addition to terms describing the crystallization itself. The latter ones contain information about equilibrium structures of possible phases and topology of the phase diagram.

It is the equilibrium phase structures and topology of the phase diagram that are studied in the present communication. Suppose that the cylindrical surface of a necessary radius containing proteins in the liquid phase already exists (i.e., there is already a cylindrical vesicle with integrated or an-

chored proteins in a liquid phase). In this case the crystallization of the protein liquid on this cylindrical surface will take place in its pure form without any consumption of energy for conformational or elastic changes. The structures and symmetries of low-symmetry phases arising under crystallization and the topology of the phase diagram are completely determined by the symmetry of a protein liquid on the cylindrical surface, which is predetermined by the anisotropic structure of the protein molecules.

The structures of possible low-symmetry phases are described by the protein density function $\rho(z,\varphi)$. The latter should be expanded in series in terms of basis functions of irreducible representations of a symmetry group of the protein liquid (Appendix).

Since on one hand one can consider crystallization of nonchiral subunits, and on the other hand the influence of the chirality on the crystallization can for some proteins be negligible, let us consider first the nonchiral case. In this case the four basis functions of the even irreducible representation $E^{(1)}$ (the representations are described in the Appendix) take the form

$$\psi_1^{(1)}=\exp\{i(kz+m\varphi)\},\quad \psi_2^{(1)}=\exp\{i(kz-m\varphi)\},$$

$$\psi_3^{(1)}=\psi_1^{(1)*},\quad \psi_4^{(1)}=\psi_2^{(1)*} \tag{1}$$

(here the asterisk means complex conjugation). The basis functions of the odd representation $E^{(2)}$ can be chosen as $\psi_i^{(2)}=n_z\psi_i^{(1)}$ $(i=1,2,3,4)$ with n_z the unit vector projection in the Oz direction. The superscript enumerates the representations (Appendix), while the subscript running from 1 to 4 counts the functions within each representation.

The density of proteins along the cylindrical surface can be expanded in terms of the basis functions of the representations $E^{(1)}$ and $E^{(2)}$. In the vicinity of the crystallization its main term takes the form

$$\rho(z,\varphi)=\rho_0+\sum_{j,k=1}^{2}\eta_j^{(k)}\{\exp(i\omega_j^{(k)})\psi_j^{(k)}(z,\varphi)+\exp(-i\omega_j^{(k)})\psi_j^{(k)*}(z,\varphi)\}, \tag{2}$$

where ρ_0 is the constant density in the liquid phase. The periodicity condition has the form $\rho(z,\varphi)=\rho(z,\varphi+2\pi)$, from which it follows that m takes integer values. The coefficients $\eta_j^{(k)}\exp(\pm i\omega_j^{(k)})$ by the basis functions in the density expansion (2) are the order parameters [5,10], hence $\eta_j^{(k)}$ and $\omega_j^{(k)}$ are their amplitudes and phases, with amplitudes being positive $\eta_j^{(k)}\geqslant 0$. The positions of the protein molecules in all possible phases correspond to the points of maxima of the density function Eq. (2).

The free energy is a function of the order parameters $\eta_j^{(k)}\exp(\pm i\omega_j^{(k)})$, which are supposed to be small and it can be expanded in a power series [10]. The free energy must be invariant under the action of the group G [5,10] thus it must be the function of some invariant combinations of the components of the order parameters $\eta_j^{(k)}$ and $\omega_j^{(k)}$. One can find a minimal set of independent invariants (referred to as the integer rational basis of invariants [11]), so that the free energy depends only on this set of invariants. The transformational properties [Eq. (A1) in the Appendix] determine the integer rational basis of invariants of the group G for each irreducible representation $E^{(1)}$ and $E^{(2)}$. In the both cases the basis consists of two invariants:

$$J_1^{(j)}=(\eta_1^{(j)})^2+(\eta_2^{(j)})^2;\quad J_2^{(j)}=(\eta_1^{(j)}\eta_2^{(j)})^2 \tag{3}$$

$(j=1,2)$. Each of them completely determines the list of possible low-symmetry phases and the possible structures of the phase diagrams, since the free energy density f should be considered as some function that depends only on the invariants appearing in the integer rational basis [Eq. (3)]: $f=f(J_1,J_2)$. One can see that the number and the structure of invariants in the case $j=1$ are the same as those in the case $j=2$. Thus the lists of phases and phase diagrams in these two cases are the same, while the structures of the phases are different.

Consider first the phases described by the order parameters transforming according to the representation $E^{(1)}$. There are four phases on the phase diagram.

(1) The liquid phase. In this phase $\eta_j^{(j)}=0$, $\rho=\rho_0$, and the symmetry group is G.

(2) The helical phase. $\eta_1^{(1)}\neq 0$; $\eta_2^{(1)}=\eta_1^{(2)}=\eta_2^{(2)}=0$ with an arbitrary value of $\omega_1^{(1)}$ [8]. The main term of the density function Eq. (2) in the helical phase in the vicinity of the phase transition has the form $\rho(z,\varphi)=\rho_0+2\eta_1^{(1)}\cos(kz+m\varphi+\omega_1^{(1)})$. Its symmetry group consists of the discrete rotations $C_{2\pi/m}$, discrete translations $T_{2\pi/k}$, and continuous helical displacements—the transformations $S_{\alpha,1}$ with $kl+m\alpha=\text{const}$ (see the Appendix). The density function $\rho(z,\varphi)$ in this phase is peaked along m helixes $kz+m\varphi+\omega_1^{(1)}=2\pi N$ (with integer $N=0,1,...,m-1$), the density function having the same maximum value $\rho_{\max}=\rho_0+2\eta_1^{(1)}$. One can see that this helical phase is not a crystalline one and there are no direct analogies to this phase either in solids or among liquid crystalline phases [9].

(3) The crystalline phase. $\eta_1^{(1)}=\eta_2^{(1)}\neq 0$; $\eta_1^{(2)}=\eta_2^{(2)}=0$, the values of $\omega_{1,2}^{(1)}$ being arbitrary. Here $\rho(z,\varphi)=\rho_0+4\eta_1^{(1)}\cos\{kz+(\omega_1^{(1)}+\omega_2^{(1)})/2\}\cos\{m\varphi+(\omega_1^{(1)}-\omega_2^{(1)})/2\}$. The symmetry operations of this phase are the discrete displacements $T_{2\pi/k}$ along Oz by the lattice parameter $2\pi/k$, the discrete rotations $C_{2\pi/m}$ around Oz through the angle $2\pi/m$; the discrete helical displacements $S_{\pi/k,\pi/m}$, and a set of the mirror planes σ_h and σ_v. The crystalline lattice is given by the points of intersections of two families of helixes: $kz+m\varphi+\omega_1^{(1)}=2\pi N_1$ and $kz-m\varphi+\omega_2^{(1)}=2\pi N_2$ with $N_1,N_2=0,1,...,m-1$ with different senses and the same pitch $h=2\pi/k$.

(4) The crystalline phase. $\eta_1^{(1)}\neq\eta_2^{(1)}\neq 0$; $\eta_1^{(2)}=\eta_2^{(2)}=0$ with arbitrary values of $\omega_{1,2}^{(1)}$. In this phase $\rho(z,\varphi)=\rho_0+2\eta_1^{(1)}\cos(kz+m\varphi+\omega_1^{(1)})+2\eta_2^{(1)}\cos(kz-m\varphi+\omega_2^{(1)})$. There are no mirror planes in this phase, while the symmetry operations $T_{2\pi/k}$, $C_{2\pi/m}$, and $S_{\pi/k,\pi/m}$ and the lattice are the same as those in phase (3).

The structures of the above phases are shown in Fig. 1.

If the crystallization is described by the order parameter transforming according to $E^{(2)}$, one gets the same list consisting of four phases with the same symmetry operations: the liquid phase 1, the helical phase $2'$ $(\eta_1^{(2)}\neq 0;\ \eta_2^{(2)}$

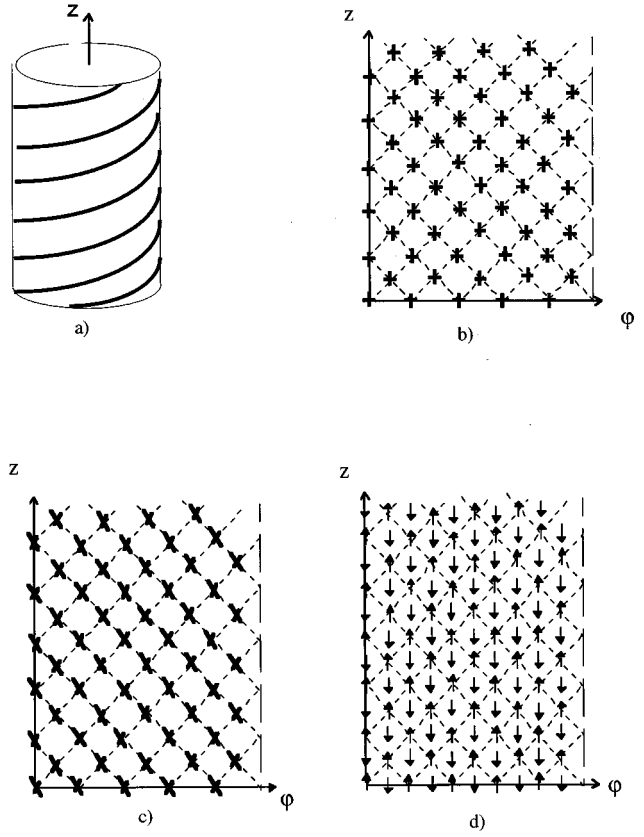

FIG. 1. The structure of some cylindrical phases. (a) The spiral phase 2. The evolvent of the cylinder ($0 \leqslant \varphi < 2\pi$) showing the structure of the crystalline cylindrical phases 3 (b) and 4 (c). The crosses are used to show schematically the local symmetry. (d) The structure of the polar phase $3'$. The extrema of the density function are located in the points of the intersections of the two families of spirals shown by the dotted lines.

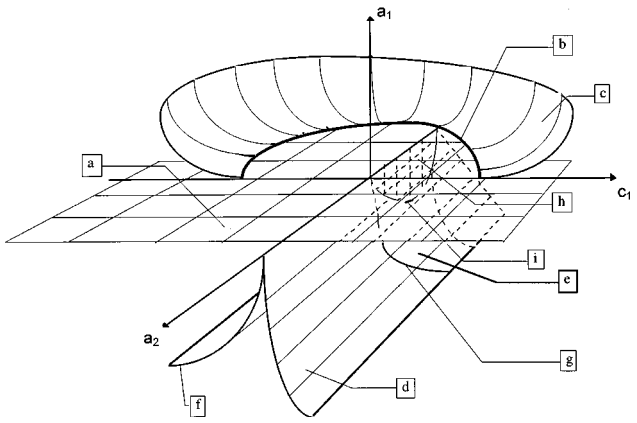

FIG. 2. The phase diagram of the crystallization on the cylinder described by the potential Eq. (4). (a) the plane (a_2, b_1). The second-order transition from the liquid phase takes place on the part of this plane limited by the line of the tricritical points (b). Behind this line the first order crystallization takes place on the surface (c). The stability region of the liquid phase is over these surfaces. The spiral phase 2 is located under the plane (a) and the surface (c) to the right of the surfaces (d), (e), and (f); the region of stability of the crystalline phase 3 is to the left of the surface (f). The phase 4 is between the surfaces (f) and (d), (e). The transition between the spiral phase 2 and the crystalline phase 4 takes place by way of the second order on the surface (d) and by way of the first order on the surface (e) separated from one another by the line of the tricritical points (g). The second-order transition between the crystalline phases 3 and 4 takes place on the surface (f) and the first-order transition takes place on its extension (which is not shown in the picture). The spiral phase 2 transforms into the crystalline phase 3 by way of the first-order transition along the segment of the plane (h) limited by the line (i) of triple points in which three phases 2, 3, and 4 are bordering.

$=\eta_1^{(1)}=\eta_2^{(1)}=0)$, and two crystalline phases $3'$ $(\eta_1^{(2)}=\eta_2^{(2)} \neq 0;\ \eta_1^{(1)}=\eta_2^{(1)}=0)$ and $4'$ $(\eta_1^{(2)} \neq \eta_2^{(2)} \neq 0;\ \eta_1^{(1)}=\eta_2^{(1)} =0)$. In contrast, the phases $2'$, $3'$, and $4'$, being polar, have structures different from those of the phases 2, 3, and 4. The representation $E^{(2)}$ describes the crystallization of the protein molecule having dipole moments along the axis of the cylinder. In this case the main term of the Oz projection of the polarization vector P_z takes the form

$$P_z(z,\varphi) \propto \sum_{j=1}^{2} \eta_j^{(2)} \{\exp(i\omega_j^{(2)})\psi_j^{(2)} + \exp(-i\omega_j^{(2)})\psi_j^{(2)*}\}. \tag{4}$$

In the lattices of phases $2'$–$4'$ the positions of the protein molecules correspond both to the points of maxima and to those of minima of the polarization Eq. (5). Thus Eq. (5) describes two times more positions than in the case of phases 2–4 with the same values of k and m [Fig. 1(d)].

The three-dimensional phase diagram in the space of the coefficients a_1, a_2, and c_1 of the free energy Eq. (A2) (Appendix) is shown in Fig. 2 [12].

Consider now the crystallization of chiral proteins on the cylindrical surface (Appendix). In order to elucidate the structural difference between the cylindrical phases in the chiral and nonchiral cases consider the chiral free energy in its simplest form, which enables us to describe crystallization of chiral proteins. To do this it is necessary to take into account the interaction of two order parameters $i=1$ and $i=2$ up to the fourth order in $\eta_1^{(j)}$ and $\eta_2^{(j)}$. The invariants of the chiral group G_{ch} are listed in the Appendix [Eq. (A3)]. The free energy takes the form

$$F = \int \left\{ \sum_{i=1}^{2} [g_{ij} I_g^{(ij)} + \lambda_{ij} I_L^{(ij)} + \alpha_{ij} I_1^{(ij)} + \beta_{ij} (I_1^{(ij)})^2] + \gamma_j I_1^{(1j)} I_1^{(2j)} \right\} dz, \tag{5}$$

where j is fixed to be either 1 or 2 and is omitted in the subsequent text. $g_i, \beta_i > 0$ and $\alpha_j = \alpha_i'(T - T_{ci})$. The differences between the transition temperatures T_{c1} and T_{c2}, and between α_1' and α_2' are supposed to be small since the weak chiral case is considered. The free energy Eq. (5) makes it possible to describe a local part of the phase diagram in the vicinity of a point where several low-symmetry phases border the liquid phase. It describes four phases: the chiral liquid phase $1''$ ($\eta_1 = \eta_2 = 0$; the symmetry group G_{ch}); two helical phases $2''$ ($\eta_1 \neq 0;\ \omega_1 = \omega_{10} + q_1 z;\ \eta_2 = 0$), and $3''$ ($\eta_2 \neq 0;\ \omega_2 = \omega_{20} + q_2 z;\ \eta_1 = 0$) and the crystalline phase $4''$ ($\eta_1 \neq 0;\ \omega_1 = \omega_{10} + q_1 z;\ \eta_2 \neq 0;\ \omega_2 = \omega_{20} + q_2 z$). The free energy Eq. (5) is minimized by choosing $q_i = -\lambda_i / 2g_i$.

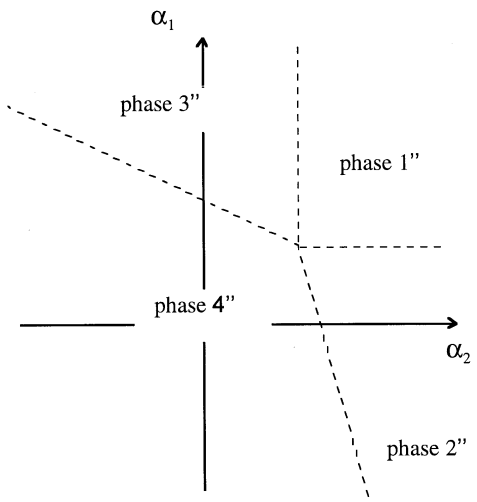

FIG. 3. One of the possible phase diagrams of the weak crystallization of chiral proteins on the cylinder in the case $\Delta>0$; $\gamma<0$. All phase transitions are of the second order. The four phases border in the multiphase point with the coordinates $\alpha_{1m}=\lambda_1^2/4g_1$, $\alpha_{2m}=\lambda_2^2/4g_2$.

In the chiral helical phases $2''$ and $3''$ the density takes the form $\rho(z,\varphi)=\rho_0+2\eta_i\cos\{(k-\lambda_i/2g_i)z+(-1)^{i+1}m\varphi+\omega_{i0}\}$ with the equilibrium values of the order parameters $\eta_i^2=-(\alpha_i-\lambda_i/4g_i)/2\beta_i$. One can see that the chiral helical phases have the same structures as those of the phase 2 in the case $j=1$ or the phase $2'$ in the case $j=2$, the sense of the helix being fixed [8].

In the chiral crystalline phase $4''$ the density takes the form $\rho(z,\varphi)=\rho_0+2\eta_1\cos\{(k-\lambda_1/2g_1)z+m\varphi+\omega_{10}\}+2\eta_2\cos\{(k-\lambda_2/2g_2)z-m\varphi+\omega_{20}\}$ with the values of the order parameters $\eta_1^2=\{\gamma(\alpha_2-\lambda_2^2/4g_2)-2\beta_2(\alpha_1-\lambda_1^2/4g_1)\}/\Delta$ and $\eta_2^2=\{\gamma(\alpha_1-\lambda_1^2/4g_1)-2\beta_1(\alpha_2-\lambda_2^2/4g_2)\}/\Delta$, where $\Delta=4\beta_1\beta_2-\gamma^2$. One can see that the protein positions in this phase are also located in the points of intersections of the two families of helixes of different senses: $(k-\lambda_1/2g_1)z+m\varphi+\omega_{10}=2\pi N_1$ and $(k-\lambda_2/2g_2)z-m\varphi+\omega_{20}=2\pi N_2$. However, in contrast to phase 4 these helixes have different pitch values $h_1=2\pi/(k-\lambda_1/2g_1)$; $h_2=2\pi/(k-\lambda_2/2g_2)$; $h_1\neq h_2$. Note that phase $4''$ corresponds to phase 4 in the nonchiral case since due to the chirality there are no mirror planes in it.

One of the possible phase diagrams containing the crystalline phase is shown in Fig. 3. The phase diagrams Figs. 2 and 3 are displayed on the planes of the coefficients of the free energy and show the regions of stability of phases in the space of the phenomenological constants. However, these constants depend on thermodynamic parameters and hence the calculated diagrams (Figs. 2 and 3) are locally isomorphous to those in the thermodynamic coordinates (temperature, pressure, concentration, and so on). Note that in contrast to classical 3D crystallization and 2D crystallization on a plane [5,6] there is no cubic invariant in the integer rational basis neither in the nonchiral nor in the chiral case. This enables the crystallization to be the second order phase transition and justifies the weak crystallization approach.

The considered system has two types of fluctuations: the order parameter fluctuations and the bending fluctuations of the cylinder. The latter kind of fluctuation depends on the cylinder rigidity and, in general, on its nature. The former one has some specific properties related to its geometry. Fluctuations are not considered in this contribution.

There is a set of 2D lattices that can be deformed into self-contained cylindrical lattices without defects. These lattices are enumerated in Ref. [3]. One should distinguish two cases. If the crystallization takes place on a hypothetical rigid cylindrical surface that cannot change its shape one should expect the formation of one of the self-contained cylindrical lattices [3] even in the case of aggregation of spherical molecules. However, when free of this constraint, these molecules must form a usual 3D lattice. In contrast, the free-of-constraint ''trapezoidal'' molecules discussed in this paper inevitably aggregate to form a cylindrical surface. The list of possible crystalline structures is determined in this case by the symmetry of the cylindrical liquid, which is predetermined by the molecular shape.

APPENDIX

The space group G of the nonchiral liquid phase on an infinite cylinder is the direct product of its point group $D_{\infty h}$ and the group of continuous translations T along the cylinder axis (the Oz axis): $G=D_{\infty h}\otimes T$. Here $D_{\infty h}=C_{\infty v}\otimes C_i$, where C_i is the inversion group while the group $C_{\infty v}$ consists of a rotation axis of an infinite order along Oz and the mirror planes passing through this axis. In International notation $D_{\infty h}=\infty/mm$; $C_i=1$; $C_{\infty v}=\infty m$; $D_\infty=\infty 2$.

The globular proteins of the tobacco mosaic virus and tubulin in the microtubules have partially α-spiral and partially β structures [1]. If the proteins have in their secondary structure α-spiral portions the protein globule manifests the chiral properties. In this case the chiral symmetry group $G_{\rm ch}$ of the protein liquid does not contain the mirror planes: $G_{\rm ch}=D_\infty\otimes T$.

The irreducible representations of the group G are constructed as the direct products of the irreducible representations of the groups $D_{\infty h}$ and T. There are two irreducible representations of the group C_i: identical (even) A_g and odd A_u. The group $C_{\infty v}$ has the identical representation A_1, the one-dimensional representation A_2, and the set of two-dimensional representations E_m. The latter have the basis functions $\exp(\pm im\varphi)$, where m is the discrete number. The translation group T has the one-parametric family of representations t_k with $\exp(\pm ikz)$ as the basis functions where k is the wave-vector projection onto the Oz axis taking continuous values and identical representation t_0. All possible irreducible representations of the group G can be obtained as terms in the expression $(A_g\oplus A_u)\otimes(A_1\oplus A_2\oplus E_m)\otimes(t_0\oplus t_k)$, containing the direct products of the direct sums of the above representations. Different crystalline orders on the cylindrical surface can be described either by one of these irreducible representations or by some kind of combination of them.

Here we consider only the case of crystallization described by the four-component representations generated by the two-dimensional representations E_m, t_k, and either A_g or A_u. The four basis functions $\psi_i^{(1)}$ $(i=1,2,3,4)$ of the even representation $E^{(1)}=A_g\otimes E_m\otimes t_k$ have the simple form Eq. (1). The basis functions of the odd representation $E^{(2)}=A_u\otimes E_m\otimes t_k$ differ from those of the $E^{(1)}$ since they change

their signs under inversion and can be chosen as $\psi_i^{(2)} = n_z \psi_i^{(1)}$ $(i=1,2,3,4)$, where n is the unit vector.

The generators of the group G are the horizontal mirror plane σ_h, one of the vertical mirror planes σ_v (one may choose the plane passing through the Ox and Oz axis), and the chiral displacement $S_{\alpha,l}=C_\alpha T_l$, which consists of the rotation C_α through the arbitrary angle α around and the displacement T_l by l along the Oz axis. Under the action of the generators the order parameters have the following transformation properties:

$$\sigma_v \eta_{1,2}^{(j)} = \eta_{2,1}^{(j)}, \quad \sigma_h \eta_{1,2}^{(j)} = (-1)^{j+1} \eta_{2,1}^{(j)}, \quad S_{\alpha,l} \eta_i^{(j)} = \eta_i^{(j)}, \tag{A1}$$

$$\sigma_h \omega_{1,2}^{(j)} = -\omega_{2,1}^{(j)}, \quad \sigma_v \omega_{1,2}^{(j)} = \omega_{2,1}^{(j)},$$

$$S_{\alpha,l} \omega_i^{(j)} = \omega_i^{(j)} + kl + (-1)^{i+1} m\alpha.$$

Transformations (A1) make it possible to determine the integer rational basis of invariants constructed with the components of the order parameters Eq. (3). The latter contains the complete information about the low-symmetry phases and the phase diagram.

In order to obtain the phase diagram of the crystallization one should expand the free energy $f=f(J_1,J_2)$ in a series in terms of the invariants J_1 and J_2. When some individual transitions are considered, depending on the order of the transition one can limit the expansion with the terms of either the fourth or the sixth degree in order parameter. However, in order to describe the whole phase diagram containing all the phases that are allowed by symmetry one should consider the expansion up to $J_1^5 \sim \eta^{10}$, the tenth-order term being isotropic:

$$f = a_1 J_1 + a_2 J_1^2 + a_3 J_1^3 + a_4 J_1^4 + a_5 J_1^5 + c_1 J_2 + c_2 J_2^2 + b_1 J_1 J_2 + b_2 J_1^2 J_2, \tag{A2}$$

where a_i, b_i, and c_i are the phenomenological coefficients, $a_5>0$. The phase diagram (Fig. 2) can be obtained by the ordinary analysis (one can find the description of the methods of this analysis in Refs. [10] and [11]).

The generators of the group $G_{\rm ch}$ are the chiral displacement $S_{\alpha,l}$ and the twofold axis C_2 along the Ox axis. In the group G the representations $E^{(1)}$ and $E^{(2)}$ are reducible in the group $G_{\rm ch}$. Each of them splits into two equivalent irreducible representations $E_i^{(1)}$ and $E_i^{(2)}$ $(i=1,2)$ with the basis functions $(\psi_1^{(j)},\psi_3^{(j)})$ for $i=1$ and $(\psi_2^{(j)},\psi_4^{(j)})$ for $i=2$. The indices $i,j=1,2$ enumerate now four irreducible representations $E_i^{(j)}$ and four corresponding order parameters amplitudes $\eta_i^{(j)}>0$ and phases $\omega_i^{(j)}$. Each of them gives rise to its own integer rational basis of invariants. Each basis consists of only one invariant $I_1^{(ij)}$. In the chiral case the mirror planes and the inversion are absent. Therefore the Lifshitz invariant $I_L^{(ij)}$ (linear in $\partial/\partial z$) can be constructed [10]. This means that the order parameters are z dependent in this case. They are described as the solutions of a differential equation, which should be obtained as the minimum condition of the free energy. The latter depends on the invariant $I_1^{(ij)}$, on the Lifshitz invariant $I_L^{(ij)}$, and for the sake of global stability of the free energy [10] one must take into account the gradient invariant $I_g^{(ij)}$ (square in $\partial/\partial z$). The invariants have the form

$$I_1^{(ij)} = (\eta_i^j)^2, \quad I_L^{(ij)} = (\eta_i^j)^2 \partial\omega_i^j/\partial z,$$

$$I_g^{(ij)} = (\partial\eta_i^j/\partial z)^2 + (\eta_i^j)^2 (\partial\omega_i^j/\partial z)^2. \tag{A3}$$

If the transition describing by two order parameters is studied (they can be fixed by, say, $j=1$, $i=1$, and $i=2$) the free energy density is constructed of the invariants (A3) built of both order parameters Eq. (5).

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[5] The theory of weak crystallization was first proposed by L. D. Landau in his paper: Zh. Eksp. Teor. Fiz. **7**, 627 (1937) [see also L. D. Landau, *On the Theory of Phase Transitions. Part 1. Collected Papers*. edited by D. Ter Haar (Pergamon Press, New York, 1965), p. 193–209]. Later this approach was developed in a series of papers. One can see the present state of the theory and the detailed references in the paper: S. A. Brazovsky, I. E. Dzialoshinsky, and A. R. Muratov, Zh. Eksp. Teor. Fiz. **93**, 1110 (1987); [Sov. Phys. JETP **66**, 625 (1987)]. In the papers mentioned the subunits of a crystallizing material—atoms or molecules were presumed to be spherical. Weak crystallization of nonspherical molecules was considered within the example of smectic mesogens in the papers E. I. Kats, V. V. Lebedev, and A. R. Muratov, Fiz. Tverd. Tela (Leningrad) **31**, 189 (1989) Sov. Phys. Solid State **31**, 652 (1989)]; Physica A **160**, 98 (1989).

[6] In the case of the weak bulk 3D crystallization (or 2D crystallization on the flat surface) the density function should be expanded in Fourier series: $\rho(r)=\Sigma\rho_k \exp(ikr)$. The amplitudes ρ_k are in this theory the order parameters describing the crystallization. Since the cubic in ρ_k free-energy term always exists in these two cases the crystallization is shown to be of the first

order everywhere except in an isolated Curie point (if the latter exists). However, fluctuations turn the isolated Curie point to a segment of a transition of a slightly first order (Ref. [5]).

[7] One should also take into account a possibility of conformational changes of proteins that can occur under crystallization and decrease the elastic energy cost.

[8] In the nonchiral case the states $\eta_1^{(1)} \neq 0;\ \eta_2^{(1)} = \eta_1^{(2)} = \eta_2^{(2)} = 0$ and $\eta_1^{(1)} = 0;\ \eta_2^{(1)} \neq 0;\ \eta_1^{(2)} = \eta_2^{(2)} = 0$ describe different domains of the same spiral phase and have the same symmetry and energy. In contrast, in the chiral case the structures of the same states differ from one another by the values of the spiral pitches $h_i = 2\pi/(k - \lambda_i/2g_i)$ and hence by the translational symmetry. Thus in the chiral case they describe different phases. The difference in their energies arises due to the Lifshits invariant.

[9] At first glance the helical phase looks like some analog of a smectic-C^* liquid crystal on a cylindrical surface. The difference is that smectic liquid crystals have a long-range orientational in-layer order in addition to the long-range translational order in the direction normal to the layers. In contrast the helical phase has no long-range orientational order within the helix.

[10] J. C. Toledano and P. Toledano, *The Landau Theory of Phase Transitions* (World Scientific, Singapore, 1988).

[11] The concept of the integer rational basis of invariants was proposed by Yu. M. Gufan, *Strukturnije Fazovije Perehodi* (Nauka, Moscow, 1982) (in Russian). One can find a description of this method in Ref. [10].

[12] The structure of the integer rational basis of invariants J_1, J_2 [Eq. (3)] is very close to that for the vector irreducible representation of the group C_{4v} (one can find it in Refs. [10] and [11]), but is not completely equivalent to the latter. While only two positive amplitudes $\eta_j^{(k)} \geqslant 0$ of the four-component order parameters appear in the basis J_1, J_2 obtained in the present contribution, the basis of the vector irreducible representation of the group C_{4v} is made up of the two-component order parameter, which can change its sign. The phase diagrams are, however, the same in these two cases, their interpretations being different. Some two-dimensional cross sections of the phase diagram in the case C_{4v} can be found in Refs. [10] and [11]. The three-dimensional C_{4v} phase diagram was briefly described by Yu. M. Gufan and E. S. Larin, Izvestija AN SSSR, Ser. Fiz. **43**, 1567 (1979) (in Russian).